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Factor $XIII_A$ Subunit Deficiency Due to a Homozygous 13-Base Pair Deletion in Exon 3 of the A Subunit Gene

Shazia Aslam, Derrick J. Bowen, Thaki Mandalaki, Renia Gialeraki, and Graham R. Standen

Molecular Haematology Unit, Bristol Royal Infirmary, Bristol (S.A., G.R.S.), Department of Haematology, University of Wales College of Medicine, Cardiff (D.J.B.), UK; Haemophilia Treatment Centre, Laikon General Hospital, Athens, Greece (T.M., R.G.)

We investigated the molecular basis of factor $XIII_A$ subunit deficiency in a Greek family. Each of the 15 exons of the A subunit gene were individually amplified by polymerase chain reaction, using previously reported oligoprimers. The proband with severe deficiency was found to have a homozygous 13-base pair deletion in the 3′ half of exon 3. The deleted sequence, extending from codons 82–86, results in a frameshift and generates a downstream termination codon in exon 4. Single strand conformation polymorphism (SSCP) analysis detected no additional mutations in the coding or consensus splice sequences of the A subunit gene. Both parents of the proband were heterozygous for the defect. Only one previous microdeletion (AG dinucleotide) has been reported in the A subunit gene, and was located at the intron B-exon 3 boundary. Further studies are necessary to determine whether this region of the gene is a "hot spot" for microdeletion mutations.

Key words: factor XIII, deletion, mutation

INTRODUCTION

Inherited factor XIII deficiency is a rare autosomal-recessive disorder which is characterized by a lifelong bleeding tendency, defective wound healing, and an increased risk of spontaneous abortion in women [1]. In plasma, factor XIII is composed of a tetramer containing two catalytic A subunits and two noncatalytic B subunits [2]. In platelets, placenta, and other tissues, it exists as a dimer of two A subunits only. Plasma factor XIII is activated by thrombin in the presence of calcium ions by cleavage of a 37-residue amino terminal peptide [3,4]. Following activation, the protein acts as a transglutaminase and catalyzes the formation of amide crosslinks between glutamine and lysine residues in a variety of proteins, including fibrin, fibronectin, and collagen [5–7]. The three-dimensional structure of the A subunit has recently been determined by X-ray crystallography [8]. The molecule consists of four domains: an N terminal β sandwich, a core domain containing the catalytic triad residues (Cys314, His373, and Asp396), and two so-called "barrel domains" of mainly β structure towards the carboxyl terminal of the monomer. Following activation, the protein undergoes sequential conformation changes which enable the macromolecule substrates access to the active site cavity.

Patients with factor XIII deficiency generally lack the catalytic A subunit, and B subunit deficiency has only been reported in rare instances [9]. The A subunit gene is located on chromosome 6 at p24–25, is 160 Kb in size, and contains 15 exons [10,11]. The gene encodes a 4-Kb mRNA transcript and a 731-residue protein product. As yet there is relatively little information on the molecular basis of factor $XIII_A$ subunit deficiency. A small number of point mutations at diverse sites [12–16], and a single individual with a homozygous dinucleotide microdeletion at the intron B-exon 3 boundary [17], have been documented. However, no patients with major gene rearrangements have been identified. In this report, we describe a patient with A subunit deficiency due to a homozygous 13-base pair deletion in exon 3 of the A subunit gene.

CLINICAL HISTORY

The pedigree of the family with factor $XIII_A$ subunit deficiency is shown in Figure 1. The female proband was

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Address reprint requests to Dr. G.R. Standen, Molecular Haematology Unit, Department of Haematology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

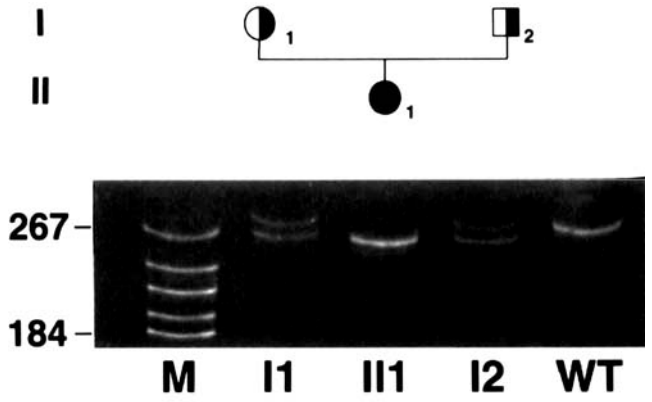

Fig. 1. PCR amplification of genomic fragment containing exon 3 of the A subunit gene. The wild-type (WT) fragment is 277 bp. Patient II1 gives a 264-bp product, indicating a homozygous 13-bp deletion. Parents I1 and I2 are heterozygous for the defect. M, molecular size markers (pBR322 digested with *Hae*III).

born in 1966 and was noted to have prolonged bleeding from the umbilical stump. Diagnosis of the coagulation disorder was made at age 7 years following an intracranial haemorrhage. No factor $XIII_A$ coagulation activity was detectable in the patient's plasma using the Berichrom photometric assay (Behringwerke AG, Marburg, Germany). Subsequently, she has received prophylactic therapy with factor XIII concentrate, which has prevented accurate measurement of factor $XIII_A$ antigen. The course of a pregnancy in 1990, covered with replacement therapy, was uncomplicated. The proband's parents originate from neighboring villages on a small Greek island, and therefore there is a strong possibility of consanguinity.

MATERIALS AND METHODS

Leukocyte DNA was isolated from peripheral blood of immediate family members by phenol extraction and ethanol precipitation [18].

Polymerase Chain Reaction Amplification

Each of the 15 exons of the factor $XIII_A$ subunit gene was individually amplified, using previously described oligonucleotide primers [12]. The oligonucleotides were synthesized using an Applied Biosystems 391A DNA synthesizer (Warrington, UK). The primer sequence (5′→3′) for the amplification of exon 3 were: sense, GATTATTTTCTTCAACCCTTG; antisense, TCTACAATGCAACCCATGG.

Polymerase chain reactions (PCR) were performed using a PREM III thermal cycler (LEP Scientific, Milton Keynes, UK). Reaction mixtures contained 50 ng of genomic DNA, 10 × reaction buffer (HT Biotechnology, Ltd., Cambridge, UK), 200 μl of each d-NTP, 0.1 μM of each primer, and sterile distilled water to a final volume of 100 μl. Following an initial denaturation step (94°C for 5 min), 0.3 units of Taq polymerase (HT Biotechnology, Ltd.) were added, the mixture was overlaid with mineral oil, and 30 cycles of amplification were performed. The PCR parameters were denaturation at 94°C for 1 min, annealing at 52°C for 1 min, extension at 73°C for 1 min, and a final primer extension step of 73°C for 10 min. Following amplification, 20 μl of PCR product were electrophoresed on a vertical 8% polyacrylamide gel in 1 × Tris/borate/EDTA (TBE) buffer for 2 hr at 300 V. The gel was stained with ethidium bromide, and the DNA bands were visualized under ultraviolet light.

DNA Sequencing

For sequencing, gel-purified double-stranded PCR product was used as a template to generate single-stranded DNA by asymmetrical PCR (primer ratio, 5:1, PCR conditions as described above). The single-stranded product was purified by gel-filtration and then sequenced using Sequinase™ (United States Biochemical Corporation, Cleveland, Ohio). The products from the sequencing reactions were electrophoresed on a standard 6% polyacrylamide, 7 M urea denaturing gel (40 cm length) at 50 mA constant current.

Single-Strand Conformation Polymorphism (SSCP) Analysis

PCR-SSCP [19] was used to screen the A subunit gene in the proband for additional mutations. Primer pairs for exons 1, 2, and 4–15 were used to amplify coding and consensus splice sequences. Following amplification, 8 μl of product were mixed with 8 μl of formamide buffer. The mixture was heated to 100°C for 5 min, cooled on ice for 5 min, and loaded on an 8% polyacrylamide gel containing 10% glycerol and 0.5 × TBE buffer, pH 5.5. Electrophoresis was performed at 300 V for 2–3½ hr at room temperature, and single-strand fragments were visualized for band shifts by silver staining.

RESULTS

The pedigree of the family with severe factor $XIII_A$ deficiency is shown in Figure 1. Amplification of a 277-bp PCR product containing exon 3 of the A subunit gene revealed that the proband (II1) had a homozygous 13-bp microdeletion (Fig. 1). Both parents (I1 and I2) were heterozygous for the defect. Amplified fragments containing exons 1, 2, and 4–15 were of normal size, and analysis of the coding sequence by PCR-SSCP revealed no band shifts indicative of additional mutations in the A subunit gene. Direct nucleotide sequencing demonstrated that the deletion extended from codons 82–86 (nucleotide C333→T345), and resulted in a frameshift and a premature stop signal (TAG) at codon 121 in exon 4 (Figs. 2, 3). The location of the deletion in the three-dimensional

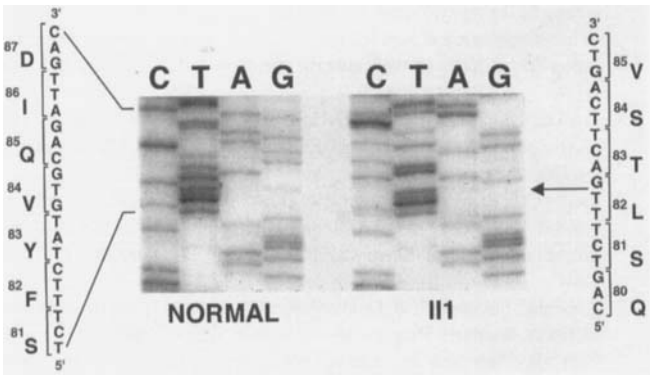

Fig. 2. Nucleotide sequence of exon 3 sense strand in proband II1. A homozygous 13-bp deletion extends from codons 82–86.

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80A   Q  S  F  Y  V  Q  I  D  F  S  R  P  Y  D  P  R  R  D  L  F
W.T.  CAGTCTTTCTATGTGCAGATTGACTTCAGTCGTCCATATGACCCCAGAAGGGATCTCTTC
      Exon 3→
80A   Q  S  L  T  S  V  V  H  M  T  P  E  G  I  S  S  G  W  N  T
MUT   CAGTCTTTGACTTCAGTCGTCCATATGACCCCAGAAGGGATCTCTTCAGGGTGGAATACG

                        Intron C
100A  R  V  E  Y  V  I  G  R  Y  P  Q  E  N  K  G  T  Y  I  P  V
W.T.  AGGGTGGAATACGTCATTGGTCGCTACCCACAGGAGAACAAGGGAACCTACATCCCAGTG
                           Exon 4→
100A  S  L  V  A  T  H  R  R  T  R  E  P  T  S  Q  C  L STP
MUT   TCATTGGTCGCTACCCACAGGAGAACAAGGGAACCTACATCCCAGTGCCTATAG

120A  P  I  V
W.T.  CCTATAGTC
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Fig. 3. Partial nucleotide and amino-acid sequence of wild-type (W.T.) and mutant (MUT) factor XIII^A subunit. The 13-bp microdeletion in exon 3 (underlined) leads to a frameshift and generates a premature stop signal (TAG) at codon 121 in exon 4 (double-underlined).

crystalline model of the A subunit monomer is shown in Figure 4.

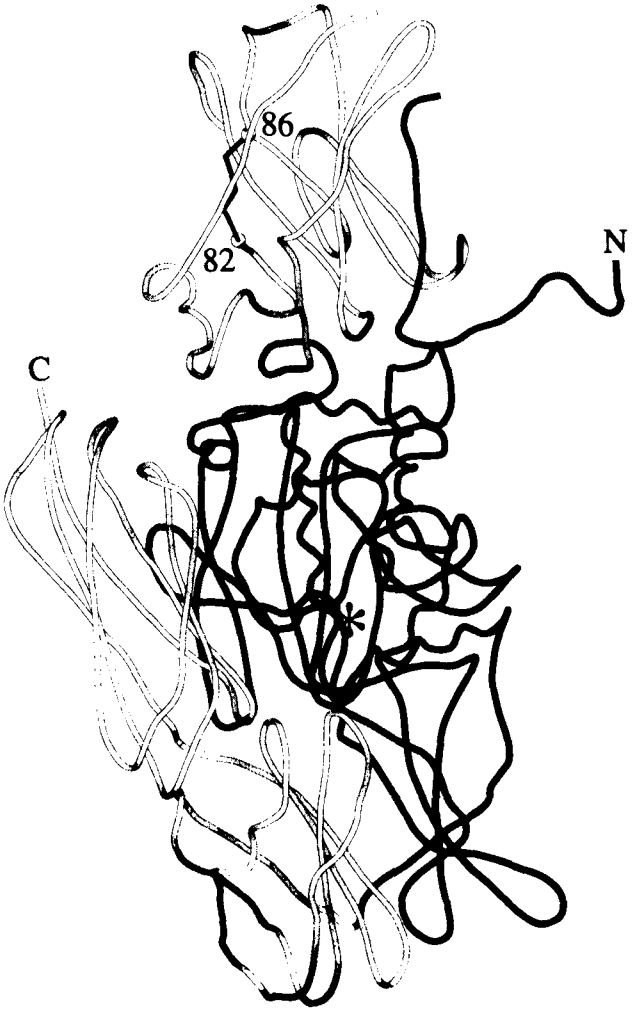

Fig. 4. View of factor XIII^A subunit structure, as determined by X-ray crystallography. Subunit is shown as a coil, and the sequence deleted (residues 82–86) is shown as a thick alpha carbon-linked segment and labelled. Asterisk marks location of active site of molecule. This illustration was generated by V.C. Yee (Department of Biochemistry, University of Washington, Seattle, WA), using the computer program MOLSCRIPT [20].

DISCUSSION

There is only limited information on the molecular basis of factor XIII deficiency. Previous studies have shown that patients with this severe bleeding disorder generally lack the catalytic A subunit protein. In this report we describe a patient with a homozygous 13-bp microdeletion in the factor XIII$_A$ subunit gene. The deletion is located in exon 3, and results in a frameshift and a downstream premature stop signal at codon 121. This region of sequence encodes part of the β sandwich domain (Glu43–Phe184) towards the N terminal of the A subunit monomer [8]. Consequently, any protein product expressed will be truncated, nonfunctional, and very likely unstable. Both parents of the proband were shown to be heterozygous for the defect. Only one previous example of a microdeletion in the A subunit gene has been reported, i.e., an AG dinucleotide deletion at the intron B-exon 3 boundary [17]. Consequently, it is conceivable that this region of the gene is a relative "hot spot" for microdeletion.

Several point mutations have been identified in the A subunit gene in factor XIII$_A$-deficient patients. Board et al. [12] have reported a single G to A transition in the last base of exon 14 in a homozygous proband, which would result in an Arg681→His substitution in the mature protein product. As the defect was adjacent to the splice junction, however, it was speculated that the base change might interfere with premessenger RNA splicing. We recently identified this mutation in a compound-heterozygous patient in combination with a nonsense mutation (Arg171→Stop) in exon 4 of the other allele [13,21]. As both patients originated from the UK, it is possible that a founder effect may account for the apparent recurrence of this mutation. A founder effect was clearly recognized in a recent study of factor XIII-deficient families in Fin-

land by Mikkola et al. [14]. Four families were homozygous for a nonsense mutation (Arg661→Stop) in exon 14, and two further families were compound heterozygous, in one case with a Met242Thr substitution in exon 6, and in the second case with a hitherto-unidentified defect.

Coggan et al. [15] recently reported three mutations in two families with factor $XIII_A$ deficiency. Two affected siblings in one family were compound-heterozygous for a Tyr441→Stop nonsense mutation in exon 11 and an Asn60→Lys missense mutation in exon 3. In the second pedigree, a Gly501→Arg missense mutation in exon 12 was identified in two heterozygous family members. Both missense mutations were constructed in cDNA clones and expressed in yeast. The Asn60→Lys mutant was transcribed but no protein was detected, suggesting extreme instability or susceptibility to proteolysis. In contrast, the Gly501→Arg mutant protein was expressed as a dimer, but showed thermal instability and accelerated degradation on storage.

We recently described a Canadian family containing 3 homozygous siblings with a candidate missense mutation in exon 14 of the A subunit gene [16]. The mutation (Leu667→Pro) was located in the beta barrel 2 domain of the A subunit molecule. Computer modelling based on three-dimensional crystallographic data predicts that the amino-acid substitution will produce gross distortion of protein conformation, disruption of three main-chain hydrogen bonds, and removal of a large hydrophobic side-chain in the core of the beta barrel 2 domain. Such changes would be expected to lead to retention and early degradation of the mutant protein product in the endoplasmic reticulum.

In summary, we report a homozygous patient with a novel 13-bp deletion in exon 3 of the factor $XIII_A$ subunit gene. Accumulating data suggest that the disorder arises from diverse genetic mechanisms. Only one previous patient has been described with a microdeletion which was situated at the intron B-exon 3 boundary [17]. Further studies are underway to determine whether deletion mutations are preferentially located in this region of the coding sequence.

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